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Effects of deletion mutations in the yeast Ces1 protein on cell growth and morphology and on high copy suppression of mutations in mRNA capping enzyme and translation initiation factor 4A

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ABSTRACT

The homologous *Saccharomyces cerevisiae* genes *CES1* and *CES4* act as high copy suppressors of temperature-sensitive mutations of Ceg1p, the yeast mRNA capping enzyme. Neither *CES1* nor *CES4* is essential for cell growth. We find that a double deletion mutant (Δ*ces1* Δ*ces4*) grows at 25–37°C, but not at 16°C. Δ*ces1* Δ*ces4* cells display gross defects in cell shape and budding even at permissive temperatures. Functional analysis of *CES1* deletion mutants defines a 145 amino acid C-terminal segment of the 915 amino acid Ces1 protein that is necessary and sufficient to complement the Δ*ces1* Δ*ces*4 cold-sensitive phenotype, to restore normal morphology and to suppress the temparature-sensitive mutant *ceg1-25*. A 147 amino acid C-terminal segment of the 942 amino acid Ces4 protein is sufficient to carry out these same functions. Within this carboxyl domain Ces1p and Ces4p are 80% identical to one another. We report isolation of *CES1* in a separate screen for high copy suppression of a temperature-sensitive mutation (A79V) of the yeast translation initiation factor Tif1p (eIF-4A). Deletion of the N-terminal 249 amino acids of Ces1p abolished *tif1-A79V* suppressor function. *CES4* on a multicopy plasmid was unable to suppress *tif1-A79V*. We surmise that whereas the carboxyl domains of Ces1p and Ces4p are functionally redundant in controlling cell morphology and in suppressing *ceg1-25*, full-length Ces1p and Ces4p evince distinct genetic interactions that are likely mediated by their N-terminal segments.

INTRODUCTION

The m^7GpppN cap has been postulated to play a role in virtually every transaction of eukaryotic mRNA, including splicing, polyadenylation, transport, translation and decay. A genetic analysis of cap function has become feasible only recently, as the mRNA capping and methylating enzymes have been purified from *Saccharomyces cerevisiae* and the genes encoding them have been identified (1,2). Yeast mRNA guanylyltransferase (capping enzyme) is a 52 kDa protein encoded by the *CEG1* gene (1). Ceg1p reacts with GTP to form a covalent enzyme–GMP intermediate. The enzyme transfers the GMP to a 5′-diphosphate-terminated RNA to form the GpppN cap structure, which is then methylated at N-7 of the cap guanosine in a reaction catalyzed by the 50 kDa *S.cerevisiae* Abd1 protein (2). The guanylyltransferase and methyltransferase activities of Ceg1p and Abd1p are essential for yeast cell growth, i.e. mutations of *CEG1* or *ABD1* that eliminate enzyme activity *in vitro* are invariably lethal *in vivo* (3–7).

We recently reported isolation of temperature-sensitive (*ts*) capping enzyme mutants and identification of multicopy suppressors of the *ceg1-ts* growth defect (8). We reasoned that capping enzyme suppressor (*CES*) genes might encode proteins that either interact with Ceg1p or impact on cap-dependent transactions *in vivo*. Four *CES* genes were identified, two of which, *CES1* and *CES4*, encode extensively similar proteins (915 and 942 amino acids respectively) of unknown function (8). We found that neither gene was essential and that a double knock-out was viable.

Other investigators have encountered *CES1* (alternatively named *ZDS1*) or *CES4* (alternatively named *ZDS2*) during genetic screening for high copy suppression of a variety of conditional growth phenotypes (9–11; D.Burke, personal communication; C.Glover, personal communication). The genetic backgrounds suppressed by *CES1* and *CES4* have no clear connection with each other. Suppression encompasses genes involved in cell polarity, transcription, cell cycle control, chromosome stability and protein modification (reviewed in 9). Elucidating the physiological role of Ces1p and Ces4p and connecting the suppressor phenotypes to a common biological process presents a fascinating challenge. In the present study we exploit the observation that a double deletion of *CES1* and *CES4* results in an inability to grow at low temperature to identify the essential structural components of Ces1p and Ces4p. We find that the C-terminal segment of either Ces1p or Ces4p is sufficent to complement the Δ*ces1* Δ*ces4 cs* growth phenotype.

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The carboxyl domain is essential for suppression of capping enzyme mutant *ceg1-25*. We also report identification of *CES1* as a high copy suppressor of a temperature-sensitive mutation in the yeast translation initiation factor eIF-4A. This suggests that *CES1* overexpression may facilitate cap-dependent RNA transactions.

MATERIALS AND METHODS

Yeast strains

The strains used in this study derive from the diploid strain YPH274 (MAT**a**/MATα *trp1Δ1/trp1Δ1 his3Δ200/his3Δ200 ura3-52/ura3-52 ade2-101/ade2-101 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1*). *CES1* was disrupted in YPH274 by a *hisG-URA3-hisG* cassette (8,12). Haploids were selected after sporulation. The Ura$^+$ haploid strain YBS101 (MAT**a** *his3 ura3 leu2 trp1 lys2 ces1*::*hisG-URA3-hisG*) carries a chromosomal deletion of *CES1*. Strains YBS102 (MAT**a** *his3 ura3 leu2 trp1 lys2*) and YBS103 (MATα *his3 ura3 leu2 trp1 lys2*) were derived from sister spores of YBS101. Plating of YBS101 on 5-FOA yielded the Ura$^-$ strain YBS105 (MAT**a** *his3 ura3 leu2 trp1 lys2 ces1*::*hisG*). *CES4* was disrupted in YBS105 by a *hisG-URA3-hisG* cassette, yielding strain YBS104 (MAT**a** *his3 ura3 leu2 trp1 lys2 ces1*::*hisG ces4*::*hisG-URA3-hisG*). After selection for cells that had lost the *URA3* marker by recombination between the *hisG* repeats we obtained the Δ*ces1* Δ*ces4* double deletion strain YBS106 (MAT**a** *his3 ura3 leu2 trp1 lys2 ces1*::*hisG ces4*::*hisG*). *CES4* was singly disrupted in YBS102 to yield the Δ*ces4* strain YBS107 (MAT**a** *his3 ura3 leu2 trp1 lys2 ces4*::*hisG*). The *CES1* and *CES4* gene disruption cassettes were described previously (8).

N-Terminal deletion mutants of *CES1*

A *Nco*I restriction site was introduced at the start codon of the *CES1* open reading frame by site-directed mutagenesis. A 1008 bp *Eco*RI–*Stu*I fragment containing the *Nco*I cleavage site was exchanged for the corresponding fragment in the 2μ plasmid pCES1-Hinc2 (8). The resulting plasmid, pCES1-N, extended from 309 bp upstream of the *CES1* start codon to 467 bp downstream of the stop codon. This clone was active in suppressing the temperature-sensitive growth defect of *ceg1-25*. N-Terminal deletion mutants of *CES1* were constructed by PCR amplification with mutagenic primers that introduced a *Nco*I restriction site and a methionine codon in lieu of the codons for Asp250, Pro358 and Ser422. The PCR products were digested with *Nco*I and *Kpn*I, then inserted into pCES1-N so as to replace a 1.38 kb *Nco*I–*Kpn*I fragment of the wild-type *CES1* gene with in-frame restriction fragments encoding serially deleted Ces1 polypeptides. The mutated genes were named according to the amino acid coordinates of their polypeptide products, i.e. *CES1(250–915)*, *CES1(358–915)* and *CES1(422–915)*. Additional N-terminal *CES1* deletion mutants were constructed by introducing *Nco*I sites with methionine codons in place of the codons for Asn529, Thr569, Asn613, His654, Lys688, Thr731, Ser771, Ala796 and Thr829. The PCR products were digested with *Nco*I and *Sac*II, then cloned into pCES1-N that had been cut with *Nco*I and *Sac*II. This yielded mutant alleles *CES1(529–915)*, *CES1(569–915)*, *CES1(613–915)*, *CES1(654–915)*, *CES1(688–915)*, *CES1(731–915)*, *CES1(771–915)*, *CES1(796–915)* and *CES1(829–915)*.

C-Terminal deletions of *CES1*

C-Terminal deletion mutants of *CES1* were constructed by excising gene fragments from pCES1-Hinc2 and inserting them into YEp24. *CES1(1–712)* contains a *Hin*cII–*Xba*I fragment extending from nt –645 to +2139. *CES1(1–626)* contains a *Hin*cII–*Hin*dIII fragment from nt –645 to +1878. *CES1(1–537)* contains a *Hin*cII–*Sac*I fragment from nt –645 to +1611.

Deletion mutants of *CES4*

CES4 deletions were constructed in stages. First, the promoter region (–500 to +3) of *CES4* was PCR amplified by using two mutagenic primers. The sense strand primer introduced a *Bam*HI site 500 bp upstream of the translation initiation codon; the antisense primer introduced a *Nco*I site at the start codon and a *Xba*I site downstream of the *Nco*I site. The 2μ *CES4* plasmid pDC1 (a gift of Desmond Clarke and Dan Burke) was used as the template for PCR amplification. The PCR product was digested with *Bam*HI and *Xba*I and the fragment inserted into pBluescript-KS+ to yield pKS-CES4(5′). Second, an *Xba*I–*Sac*II fragment extending from nt +2818 to +3718 of *CES4* was inserted into pKS-CES4(5′) to yield the plasmid pKS-CES4(5′)(3′). Third, the *CES4* coding region was PCR amplified with mutagenic primers that introduced a *Nco*I restriction site and a methionine codon in lieu of the codons for Glu707, Glu796 and Ser841. The PCR products were digested with *Nco*I and *Xba*I and then inserted into pKS-CES4(5′)(3′). Finally, *Xho*I–*Sac*I fragments containing the truncated *CES4* genes were excised from the Bluescript-based plasmids and inserted into YEp24.

Suppression of a temperature-sensitive mutation of eIF-4A

Yeast strain SS13-3A (MAT**a** *his3 ade2 leu2 trp1 ura3 tif1*::*HIS3 tif2*::*ADE2*) is deleted at the two chromosomal loci (*TIF1* and *TIF2*) encoding the translation factor eIF-4A (13). Viability of SS13-3A is dependent on maintenance of an extrachromosomal copy of *TIF1*. The strain used in this study contains plasmid pSSC120 (*CEN LEU2 tif1-A79V*), which bears a temperature-sensitive *tif1* allele (14). This strain grows at 25 but not at 37°C. The conditional phenotype is caused by a single coding change resulting in substitution of valine for alanine at residue 79 of eIF-4A (14). SS13-3A/pSSC120 was transformed with 2μ *URA3* plasmids containing *CES1*, *CES4* and various deletion mutants of *CES1* and *CES4*. A control transformation was performed with plasmid YCplac33-TIF1 (*CEN URA3 TIF1*), which contains the wild-type *TIF1* gene. Ura$^+$ transformants were selected at 25°C. Single colonies were amplified and then streaked onto synthetic medium lacking uracil (SD Ura$^-$). The plates were incubated at 25 and 37°C.

RESULTS

Deletion of *CES1* and *CES4* elicits a cold-sensitive growth phenotype

Single deletion mutants Δ*ces1* and Δ*ces4* formed wild-type sized colonies at 30°C. The double deletion mutant strain Δ*ces1* Δ*ces4* was viable at 30°C, but the colony size was smaller (Fig. 1). Conditional phenotypes were sought by testing cell growth at other temperatures. The Δ*ces1* and Δ*ces4* strains grew at 37, 25 and 16°C (Fig. 1 and data not shown). The Δ*ces1* Δ*ces4* double mutants formed colonies at 37, 30 and 25°C, but failed to grow

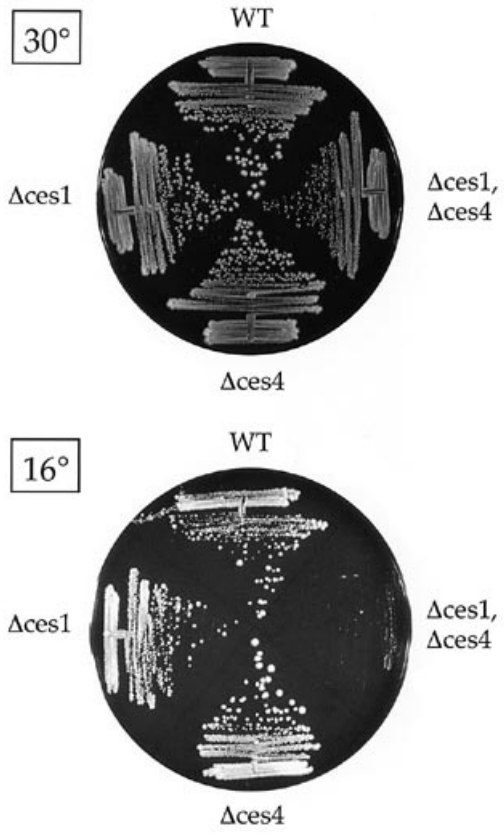

Figure 1. Disruption of *CES1* and *CES4* elicits a cold-sensitive growth phenotype. The singly deleted Δ*ces1* and Δ*ces4* strains and the Δ*ces1* Δ*ces4* double deletion strain were constructed as described in Materials and Methods. Strain YBS103 was used as the *CES1 CES4* wild-type (WT) control. The cells were streaked on YPD medium. The plates were photographed after incubation for 3 days at 30°C or 6 days at 16°C.

at 16°C (Fig. 1). Hence, the loss of both gene products confers a cold-sensitive (*cs*) growth phenotype.

Gross morphological abnormalities caused by deletion of *CES1* and *CES4*

Microscopic examination of Δ*ces4* cells grown to mid log phase in liquid culture at permissive temperature revealed unbudded and budded cells (Fig. 2) that were indistinguishable in appearance from wild-type cells. Δ*ces1* cells displayed a mild defect in morphogenesis in that they tended to form elongated buds (Fig. 2). In contrast, Δ*ces1* Δ*ces4* cells were grossly misshapen (Fig. 2). Large mother cells elaborated very long buds that were punctuated by multiple constrictions at regular intervals. The distal tip of the bud projection was sometimes malformed. A few cells had schmoo-like protuberances. Bi and Pringle (9) and Yu *et al.* (11) noted similar morphological defects in Δ*zds1* Δ*zds2* deletion strains (equivalent to Δ*ces1* Δ*ces4*).

Complementation of Δ*ces1* Δ*ces4* by either *CES1* or *CES4*

Introduction of the *CES1* gene on a centromeric or multicopy plasmid complemented the *cs* phenotype of the Δ*ces1* Δ*ces4* strain and restored growth at 16°C (Fig. 3). Cells transformed with the vectors alone did not grow. *CES4* on a multicopy plasmid also complemented the *cs* phenotype (Fig. 3). (*CEN CES4* was not tested.) Microscopic examination of the transformants showed that the morphological defect was complemented in parallel with the growth defect (not shown). We conclude that Ces1p and Ces4p carry out overlapping functions that are conditionally essential for cell growth.

Structure–function analysis of Ces1p

Ces1p is a hydrophilic 915 amino acid protein (8). The amino acid sequence of Ces1p is uninformative with respect to its potential function. Complementation of the cold-sensitive growth phenotype of the Δ*ces1* Δ*ces4* strain affords a simple test of *CES1* function that we applied to the analysis of *CES1* alleles containing N- and C-terminal deletions. All *CES1* mutants were cloned into a 2μ vector under transcriptional control of the natural *CES1* promoter. This allowed us to test in parallel the ability of the mutant alleles to function as multicopy suppressors of a temperature-sensitive mutation in the *CEG1* gene encoding the mRNA capping enzyme, this being the basis for our original isolation of *CES1*.

Deletion of the N-terminal 249 amino acids of Ces1p had no impact on its ability to complement Δ*ces1* Δ*ces4* growth at 16°C (Fig. 4A). Nine additional incremental deletions showed that the N-terminal 770 amino acids could be eliminated with no effect on complementation activity. The *CES1(771–915)* allele also corrected the morphogenesis defect of the Δ*ces1* Δ*ces4* mutant (not shown). Hence, a C-terminal 145 amino acid fragment of Ces1p was fully functional by these two criteria. The *CES1(796–915)* allele, encoding a 120 amino acid C-terminal fragment, was partially active in complementing Δ*ces1* Δ*ces4* growth at 16°C, whereas *CES1(829–915)* was incapable of *cs* complementation (Fig. 4A).

The allele *CES1(1–712)*, which lacks the C-terminal 203 amino acids, was inactive in the complementation assay, as were two more extensively truncated alleles, *CES1(1–626)* and *CES1(1–537)*, from which 289 and 378 C-terminal residues were deleted respectively (Fig. 4A). We conclude from these results that: (i) the N-terminal 85% of Ces1p is dispensable for the essential function of *CES1* at 16°C; (ii) the C-terminal polypeptide segment is necessary and sufficient for this function.

Effects of *CES1* mutations on capping enzyme suppression

ceg1-25 is a temperature-sensitive mutation of the yeast mRNA capping enzyme (8). *ceg1-25* cells are able to form colonies at the restrictive temperature (37°C) when they are transformed with *CES1* on a multicopy plasmid (8). Truncated versions of *CES1* were introduced into *ceg1-25* and the transformants tested for growth at 37°C. We found that *CES1(688–915)* was as active as the full-length gene in suppressing *ceg1-25*. Alleles *CES1(731–915)* and *CES1(771–915)* were partially active, whereas *CES1(796–915)* and *CES1(829–915)* were non-functional (Fig. 4A). C-Terminal deletions of *CES1* abrogated suppressor function. We conclude that the N-terminus of the Ces1p protein is dispensable for suppressor function, just as it is for complementation, and that the C-terminus is necessary and sufficient for suppression. The cut-off point for loss of suppressor activity by incremental N-terminal deletion (residue 771) was only 15 amino acid residues proximal to the transition point, beyond which complementation function was lost (residue 796) (Fig. 4A). We infer that the two phenotypic manifestations of *CES1* function are tightly linked within the C-terminal protein domain.

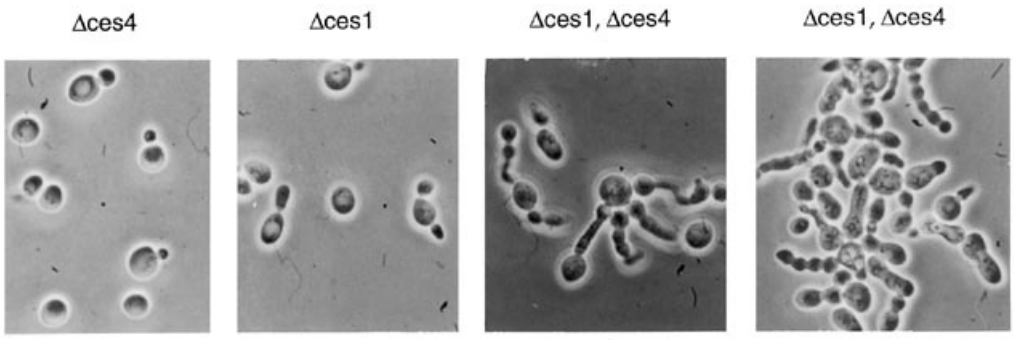

Figure 2. Aberrant morphology of Δ*ces1* Δ*ces4* cells. The singly deleted Δ*ces1* and Δ*ces4* strains and the Δ*ces1* Δ*ces4* double deletion strain were grown in liquid cultures of YPD medium at 25°C. Cells were examined by phase contrast microscopy. Two diffferent microscopic fields of Δ*ces1* Δ*ces4* cells are shown.

Structure–function analysis of *CES4*

Deleted alleles of *CES4* under transcriptional control of the natural *CES4* promoter were cloned into a 2μ plasmid vector and tested for complementation and suppressor activities *in vivo*. We found that elimination of 706 or 795 amino acids from the N-terminus of the 942 amino acid Ces4p protein had no effect on complementation, whereas deletion of 840 residues abolished complementation activity (Fig. 4B). The truncated allele *CES4(796–942)* corrected the Δ*ces1* Δ*ces4* morphogenesis defect (not shown). *CES4(707–942)* and *CES4(796–942)* were partially active as high copy suppressors of *ceg1-25*, i.e. the colonies formed at 37°C by the mutant alleles were smaller in size than those of wild-type *CES4* transformants. Suppression was eliminated by further truncation to residue 840. These results show that a 147 amino acid C-terminal fragment of Ces4p was functional in both assays.

A conserved C-terminal domain of Ces1p and Ces4p

Ces1p and Ces4p display 50–80% identity to each other within a series of conserved sequence blocks that span the entire length of both proteins (8). Yet only the C-terminal sixth of either polypeptide is relevant to the overlapping function of these proteins in cell growth at reduced temperatures and to their ability to compensate for diminished capping enzyme function at high temperature. Alignment of the C-terminal domains of Ces1p and Ces4p highlights a 101 amino acid segment (positions 804–904 in Ces1p and 816–916 in Ces4p) within which 82 of the residues are identical and five more positions are occupied by conserved non-identical sidechains (Fig. 4C).

CES1 is a high copy suppressor of a temperature-sensitive eIF-4A mutation

Cap-dependent recruitment of mRNA to the 40S ribosome is mediated by translation initiation factor eIF-4F, which binds to the methylated cap structure. Mammalian eIF-4F consists of three subunits: eIF-4E (cap binding protein), eIF-4G and eIF-4A (reviewed in 17). Mammalian eIF-4A is an RNA-dependent ATPase that, together with eIF-4B, functions to unwind RNA secondary structures during ribosomal scanning for the AUG initiation codon (16). *Saccharomyces cerevisiae* genes *TIF1* and *TIF2* encode identical eIF-4A proteins. Yeast strain SS13-3A/pSSC120 contains chromosomal deletions in the *TIF1* and *TIF2* genes encoding translation initiation factor eIF-4A (13).

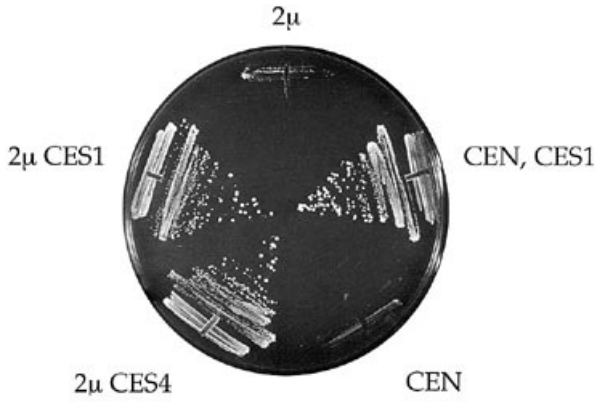

Figure 3. Complementation of the Δ*ces1* Δ*ces4* cold-sensitive growth phenotype. The Ura$^-$ Δ*ces1*,Δ*ces4* strain was transformed with *CES1*- or *CES4*-containing plasmids p360-CES1 (*CEN CES1*), pDC1 (2μ *CES4*) and pCES1-Hinc2 (2μ *CES1*) and with vector controls pSE360 (*CEN*) and YEp24 (2μ). Ura$^+$ transformants were selected at 30°C. Single colonies were amplified and then streaked onto synthetic medium lacking uracil (SD Ura$^-$). The plates were photographed after incubation for 12 days at 16°C.

Viability is sustained by maintenance of a temperature-sensitive allele *tif1-A79V* on a *CEN LEU2* plasmid (pSSC120). Coppolecchia *et al.* (13) have reported isolation of multicopy suppressors of the temperature-sensitive growth phenotype by transformation of the *tif1-A79V* strain with a 2μ plasmid library of yeast genomic DNA. Two distinct genes, *STM1* and *STM2*, restored growth at the non-permissive temperature (37°C). Sequencing of the *STM1* gene (also called *TIF3*) revealed that it encodes the yeast homolog of mammalian translation initiation factor eIF-4B (13,17).

Although the 2μ *STM2* clone was not characterized in the previous study, it has since been sequenced and found to contain *CES1*. To confirm that *CES1 per se* was capable of suppression we transformed the *tif1-A79V* strain with the same 2μ *CES1* plasmid used in the experiments described above. Suppression was tested by streaking cells onto agar plates and scoring for colony formation at 37°C. Transformation with a 2μ plasmid containing wild-type *TIF1* served as a positive control for complementation of *tif1-A79V* growth at 37°C (Fig. 5). Transformation with the 2μ vector alone could not sustain growth at 37°C. We found that a 2μ plasmid with the full-length *CES1* gene suppressed the *tif1-A79V* growth defect

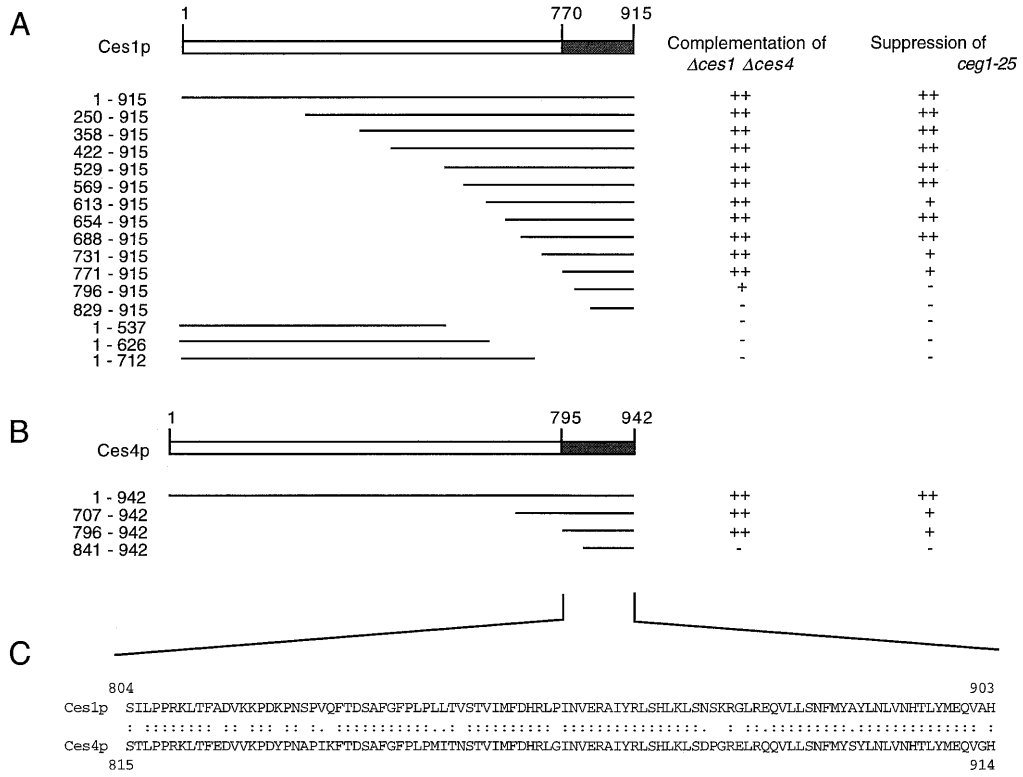

Figure 4. Complementation of Δ*ces1* Δ*ces4* and suppression of *ceg1-25* by deletion mutants of *CES1* and *CES4*. Complementation. The *ura3* Δ*ces1* Δ*ces4* strain was transformed with 2μ *URA3* plasmids bearing the indicated alleles of *CES1* (**A**) and *CES4* (**B**). Ura$^+$ transformants were selected at 30°C. Three individual isolates were patched to SD Ura$^-$ plates at 30°C, after which they were tested for growth at 16°C. Complementation of cold-sensitive growth was gauged after incubation for 12 days at 16°C. Cells transformed with full-length wild-type alleles of *CES1* and *CES4* were scored as ++ growth. Cells transformed with the YEp24 vector failed to form colonies at 16°C; this was scored as –. Alleles that supported intermediate growth at 16°C were scored as + . Suppression. A *ceg1-25* strain was transformed with 2μ *URA3* plasmids bearing the indicated alleles of *CES1* (**A**) and *CES4* (**B**). Ura$^+$ transformants were selected at the permissive temperature (25°C). Three individual isolates were patched to SD Ura$^-$ plates at 25°C, after which they were streaked and incubated for 6 days at the non-permissive temperature (37°C). The strength of suppression was scored by comparison with the wild-type *CES1* and *CES4* plasmids (++) and the YEP24 vector (–). (**C**) The amino acid sequences of a 101 amino acid segment of the essential carboxyl domains of Ces1p and Ces4p are aligned to each other. Identical residues are denoted by : . Similar residues are denoted by . .

(Fig. 5). However, 2μ clones containing deletion mutants *CES1(654–915)*, *CES1(688–915)* and *CES1(731–915)* were incapable of suppressing *tif1-A79V* (Fig. 5). *CES1(250–915)*, *CES1(529–915)*, *CES1(569–915)* and *CES1(613–915)* were also unable to suppress *tif1-A79V* (not shown). Hence, the N-terminus of Ces1p, though dispensable for suppression of *ceg1-25*, was required for suppression of *tif1-A79V*. The C-terminus of Ces1p was also required, insofar as 2μ plasmids containing the deletion mutants *CES1(1–712)*, *CES1(1–626)* and *CES1(1–537)* were unable to suppress *tif1-A79V* (not shown). A 2μ plasmid containing the full-length *CES4* gene was unable to suppress the *tif1-A79V* mutation (not shown). Deletion mutants *CES4(707–942)* and *CES4(796–942)* were also inactive as *tif1-A79V* suppressors (not shown). We surmise that *CES1* and *CES4* are functionally redundant with respect to some, but not all, genetic functions. To eliminate *CES1* and *CES4* promoter differences as variables we constructed 2μ vectors in which expression of the *CES1*, *CES1(688–915)* and *CES4(796–942)* alleles was driven by the strong constitutive *TPI* promoter. The *TPI–CES1* plasmid complemented the Δ*ces1* Δ*ces4* growth defect and suppressed both *ceg1-25* and *tif1-A79V* (not shown). The *TPI–CES1(688–915)* and *TPI–CES4(796–942)* plasmids complemented Δ*ces1* Δ*ces4* and suppressed *ceg1-25*, but did not suppress *tif1-A79V* (not shown).

DISCUSSION

CES1 and *CES4* encode structurally similar proteins with overlapping functions in cell morphology. In the absence of Ces1p and Ces4p yeast cells form abnormally long bud appendages. The microscopic appearance of Δ*ces1* Δ*ces4* cells suggests that cytokinesis does not proceed to completion after emergence of the first bud from the mother cell and that new rounds of budding ensue from the same site on the mother cell, each round accompanied by incomplete cytokinesis; hence, the regularly spaced constrictions. Insofar as the loss of Ces1p and Ces4p causes overzealous budding, we infer that these proteins normally exert a negative influence on bud formation. Bi and Pringle (9) and Yu *et al.* (11) reported similar defects in cell shape in Δ*zds1* Δ*zds2* deletion strains (equivalent to Δ*ces1* Δ*ces4*).

We have exploited the observation that Δ*ces1* Δ*ces4* cells have a cold-sensitive growth phenotype to identify the minimum functional domains of Ces1p and Ces4p. A 145 amino acid carboxyl domain of Ces1p or a 147 amino acid carboxyl domain of Ces4p suffice in

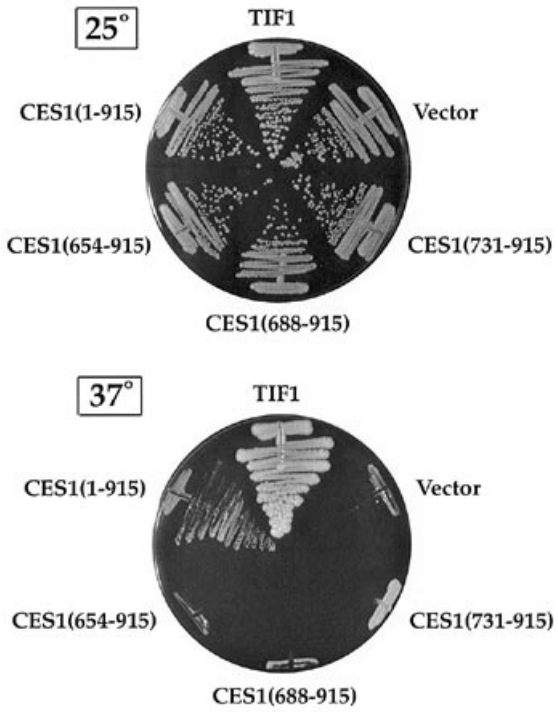

Figure 5. *CES1* suppresses a temperature-sensitive mutation of the translation initiation factor eIF-4A. A *tif1-A79V* strain was transformed with 2μ *URA3* plasmids bearing the indicated alleles of *CES1*. Control transformations were performed with the 2μ *URA3* vector (YEp24) and with a *CEN URA3* plasmid containing the wild-type *TIF1* gene. Ura^+ transformants were selected at 25°C. Single colonies were amplified and then streaked onto SD Ura^- medium. The plates were photographed after incubation for 4 days at 25°C or 6 days at 37°C.

complementing *cs* growth and correcting the morphological defect of Δ*ces1* Δ*ces4* cells. It is remarkable that the N-terminal 85% of both proteins are dispensable for these functions.

Bi and Pringle (9) identified *ZDS1* (*CES1*) as a high copy antagonist of the ability of overexpressed *CDC42* to suppress a temperature-sensitive allele of *cdc24*. Cdc42p is a ρ-type GTPase essential for establishment of cell polarity during budding (18). Cdc24p functions as a guanine nucleotide exchange factor for Cdc42p (19). Bi and Pringle suggest that the negative regulatory effects of Ces1p on budding are mediated through genetic interactions between Ces1p and Cdc42p at the bud site (9). This model is supported by immunolocalization of overexpressed Ces1p protein at the bud site (9).

It is tempting to speculate that the C-terminal domains of Ces1p and Ces4p interact directly with Cdc42p and thereby affect interactions between Cdc42p and other components of *CDC42*-dependent signal transduction pathways. Ces1p and Ces4p could either: (i) promote the interactions of some effectors with Cdc42p, e.g. those that limit or negatively regulate Cdc42p activity; (ii) destabilize Cdc42p interaction with positive effectors of Cdc42p; (iii) do both. In preliminary experiments using the two-hybrid system we have not been able to detect a binary interaction between the carboxyl domain of Ces1p and Cdc42p (B.Schwer, unpublished results). Thus it is possible that Ces1p and Ces4p impact on budding via higher order protein–protein interactions.

A central issue regarding *CES1* and *CES4* is whether the high copy suppressor screens that led to identification of these genes illuminate physiological properties of the two proteins and, if so, how. Both *CES1* and *CES4* are allele-specific high copy suppressors of mutations in the mRNA capping enzyme (8). We have now shown that capping enzyme suppressor function is mediated by the same protein domain responsible for normal cell morphology and low temperature growth. This outcome was by no means self-evident. For example, another capping enzyme suppressor, *CES2*, was found to be identical to *ESP1*, an essential gene required for nuclear division. Deletion analysis showed that the 1573 amino acid protein Esp1p is composed of two distinct functional domains (8). The C-terminal portion of Esp1p is essential for cell growth, but dispensable for suppression of mutations in capping enzyme. The N-terminus of Esp1p does not support cell growth, but does have capping enzyme suppressor activity (8).

What remains elusive is a biochemical explanation for the ability of the short carboxyl domain of Ces1p and Ces4p to perform two seemingly unrelated functions, i.e. ensuring proper budding and suppressing a capping enzyme mutation. It is conceivable that the carboxyl domains of Ces1p and Ces4p suppress the capping enzyme mutants by virtue of their effects on GTP binding proteins that mediate cap-dependent or cap-facilitated RNA transactions *in vivo*. Translation initiation and RNA transport are regulated by GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GNEFs) that are specific for the pertinent GTPase, just as bud emergence and bud site selection in yeast are regulated by GAPs and GNEFs specific for the GTPases Cdc42p and Bud1p respectively (20–23). We speculate that Ces1p and Ces4p, which may normally regulate Cdc42p, can also impact on other GTPase-regulated pathways when they are expressed at supra-physiological levels. The idea that capping enzyme suppression by *CES1* and *CES4* is mediated via effects on a GTPase pathway is bolstered by the finding that capping enzyme mutations are suppressed by *BUD5*, a non-essential gene involved in bud site selection, which encodes a GNEF for the Bud1p GTPase (8,23).

Our present findings emphasize the overlapping functions of the Ces1p and Ces4p carboxyl domains in complementing Δ*ces1* Δ*ces4* and suppressing *ceg1-25*. Yet, data presented here and elsewhere suggest that *CES1* and *CES4* are *not* functionally redundant in every genetic assay. The present study documents a new activity of *CES1* in suppressing a mutation of eIF-4A, a translation initiation factor that functions in concert with other translation factors to effect binding of capped mRNAs to the 40S ribosome and transit of the ribosome to the initiator AUG. There is at least a conceptual link between the activities of Ces1p in compensating for mutations in cap formation and cap-dependent translation initiation. Yet, the structural requirements for the two suppressor functions appear to be distinct, insofar as *tif1-A79V* suppression was abolished by N-terminal *CES1* deletions that had no effect on *ceg1-25* suppression. *CES4*, which suppressed *ceg1-25* at 37°C, was not able to suppress *tif1-A79V* at 37°C.

Bi and Pringle (9) noted that *ZDS2* (*CES4*) is inactive in the high copy suppression assay in which *ZDS1* was isolated, i.e. *ZDS2* does not antagonize suppression of a *cdc24-ts* mutation by multicopy *CDC42*. Yu *et al*. (11) isolated *ZDS2* in a screen for high copy suppression of the temperature-sensitive growth phenotype of a *sin4* null mutant; *ZDS1* is non-functional in this suppression assay. Overexpression of Ces1p and Ces4p may elicit distinctive effects on the basis of differing affinities of their N-terminal domains for different cellular effector molecules. Indeed, it is entirely possible, if not likely, that Ces1p and Ces4p perform functions (ancillary to budding) to which the conserved

N-terminal domains do contribute. The putative targets of overexpressed Ces1p and Ces4p need not be limited to GTPases. Indeed, it is noteworthy that several groups have isolated *CES1* or *CES4* by screening for high copy suppression of protein kinase mutants (10,11; C.Glover, personal communication). Our collection of Ces1p and Ces4p mutants may prove useful in delineating the structural requirements for the rest of the 'Zillion Different Suppressor' activities of these two proteins.

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